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(56) Documents Cited
GB 1323800 A EP 0385335 A2 WO 91/06292 A1
WO 85/03225 A1 US 4957881 A US 4929774 A
Chemical Abstracts 119:56200 & DE-4141351-A
Chemical Abstracts 113:65273 & JP-020049091
Chemical Abstracts 109:215833 & Khim. Prir.Soedin. 4, pages 524-9 (1988) Chemical Abstracts 107: 161484 & Acta Pharm. Hung. 57(3-4), pages 153-8 (1987)

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(54) Pharmaceutically-active carotenoid antioxidants

(57) Lutein and other hydrophillic carotenoids are disclosed for use in treatment by therapy or prophylaxis of diseases having an oxygenation mechanism. The carotenoids disclosed are especially useful in treatment of coronary heart disease and may be combined with eg aspirin.

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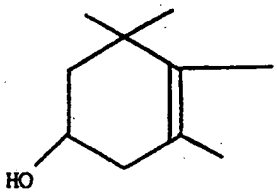
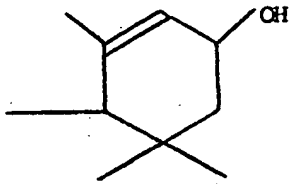
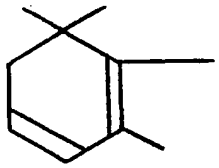
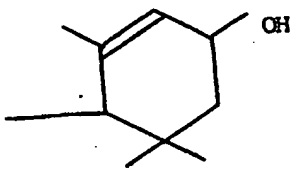
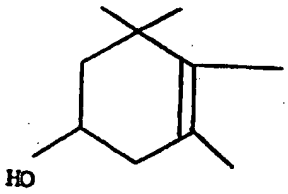
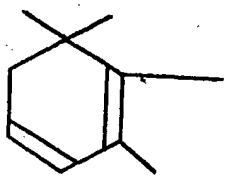
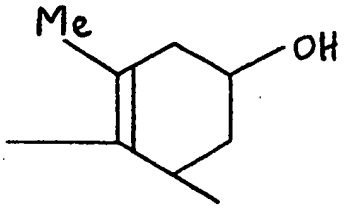
Basic Structure

Compound	R_1	R_2
Lutein		

Anhydrolutein:-

*  

** 

***  

Pharmaceutically-active antioxidants

The present invention relates to hydroxy carotenoid antioxidants (HCA) active against reactive oxygen species (ROS) and free radicals which cause oxidative damage to lipids, lipoproteins, proteins and DNA. The invention is particularly but not exclusively concerned with carotenoid antioxidants for use in the treatment by therapy or prophylaxis of coronary heart disease (CHD) in particular by a mechanism involving antioxidative protection of lipoproteins, especially low density lipoproteins (LDL).

Antioxidant nutrients such as vitamin E, vitamin C and betacarotene are considered to have important potential in the prevention of several human diseases, in particular cardiovascular and cerebrovascular disease, some forms of cancer, diabetes, rheumatoid arthritis, Parkinson's disease, Down's syndrome, Alzheimer's disease and several other age-related disorders such as cataracts.

At the present time there is much theoretical evidence that suggests a role for the activated chemicals known as free radicals in the pathogenesis of these diseases, and intensive research worldwide is being directed to the investigation of mechanisms. There is furthermore a large body of epidemiological evidence that indicates that in populations receiving large amounts of antioxidant nutrients, the risk of disease is lowered by amounts that

are statistically significant.

Carotenoids are known to act as anti-oxidants in vitro. To date there have been few practical proposals for a formulation for use as an antioxidant. One such is Redoxon Protector sold in the UK by Roche Nicholas Consumer Health Care and comprising beta carotene combined with vitamins C and E put up as one-a-day capsules.

European Patent Application No 0 385 335 discloses stabilised fat-soluble vitamin compositions which have a wide range of applications, such as in medicines and food additives. The disclosed compositions basically comprise a fat-soluble vitamin in combination with a carotenoid compound as stabiliser.

In addition, various food additives and compositions comprising a carotenoid are disclosed in UK Specifications Nos 1081104A and 918399A, Japanese Patent Publications Nos 57-003861A, 41-58749A and JP 60-054647A and US Patent 4239782.

To date the only extensive research work on carotenoids has been carried out with betacarotene. That compound is available very cheaply commercially, and is used as a permitted colourant for foods.

In a paper by Sies et al Ann. New York Acad. Sci (1992)

669, 7 to 20 (page 14) it is stated that the antioxidant activity of betacarotene would be shared by other carotenoids, but no data is given. It has always been assumed that because of their structure the hydroxy carotenoids would be similar to betacarotene in activity.

As will be appreciated from the Examples which follow the invention approaches the prevention or cure of disease involving an oxidation mechanism such as oxidation of lipids, lipoproteins, proteins and DNA from a novel standpoint based on the use of at least one hydrophilic carotenoid compound.

It will further be appreciated that the invention provides hydrophilic carotenoids, in particular lutein, which are effective in the treatment by prevention or cure of coronary heart disease.

It will furthermore be appreciated that the present invention provides a novel treatment to prevent the onset of coronary heart disease based on the use of at least one hydrophilic carotenoid, especially lutein.

Within the above context it has now found surprisingly that by selecting a particular type of carotenoid, namely a hydrophilic carotenoid, especially a mono- or di-hydroxy carotenoid such as lutein, or an ester thereof, significant and better antioxidant properties can be brought into play

within any particular preventative or curative context. This is surprising since if lutein or the like has identical properties to betacarotene it would be thought according to the teaching of Sies et al, that lutein or the like should have equal activity. However, this has been shown manifestly not to be the case.

Accordingly, the present invention in one aspect provides a compound for use as a pharmaceutical or food supplement, particularly in the prevention or cure of disease involving an oxidation mechanism such as oxidation of lipids, lipoproteins, proteins or DNA, which compound comprises a hydrophilic carotenoid or a mixture thereof.

In carrying out the invention it is preferred to use a mono-or di-hydroxy carotenoid or an ester thereof, a ketonic carotenoid, or a mixture of the same. As will be appreciated by the skilled man, ketonic carotenoids can exist in a keto/enol equilibrium, thus effectively or potentially being themselves hydroxy carotenoids. The compounds of the invention are especially useful in the prevention or treatment of cardiovascular or cerebrovascular disease, cancer, diabetes, rheumatoid arthritis, Parkinson's disease, Down's syndrome, Alzheimer's disease or cataracts or other age related changes.

In carrying out the invention there may be used a compound

as defined in its free form or in the form of an ester. Typical such esters are C_1 to C_6 esters e.g. ethyl esters, and esters with long chain fatty acids e.g. stearic, palmitic and linoleic esters or naturally occurring esters such as lutein ester from certain plants e.g. marigold.

In another aspect the invention provides a food supplement or pharmaceutical composition, which composition comprises as an active agent a hydrophilic carotenoid, especially a mono- or di-hydroxy carotenoid such as lutein, or an ester thereof, a ketonic carotenoid, or a mixture of the same, together with a food supplement or pharmaceutically-acceptable diluent or carrier.

Such a composition may be in bulk form or, more preferably, unit dosage form. Thus, for example, the composition may be formulated as a tablet, capsule, powder, solution or suspension.

Compositions in accordance with the invention may be prepared using the carotenoid or ester active agent in accordance with conventional food supplement or pharmaceutical practice. The diluents, excipients or carriers etc. which may be used are well known in the formulation art and the form chosen for any particular regimen will depend on the given context and the formulator's choice.

In carrying out the invention, for example, in formulating the compositions of the invention the active agent may be a mono-hydroxy carotenoid, a di-hydroxy carotenoid or a ketonic carotenoid per se or an ester thereof, preferably a carotenoid as described below, ~~which description includes:~~

The accompanying drawings show formulae for certain of the carotenoids more fully described in Data for Biochemical Research, Edited by R.M.C DAWSON et al., 2nd Edition, 1969, Clarendon Press: Oxford pages 327 to 333 as follows:

Mono-hydroxy

α-Cryptoxanthin

β-Cryptoxanthin

Anhydrolutein

(The structure of anhydrolutein is not fully documented, but it is thought to be one of the following, namely:

3,4-dehydro-3'-monohydroxy-alpha-carotene (*)

2',3'-dehydro-3-monohydroxy-alpha-carotene (**) or

3'-hydroxy-3,4-dehydro-beta-carotene (***)

- see the accompanying drawings which show formulae therefor identified by *'s)

Di-hydroxy

Lutein - namely, 3,3'-dihydroxy-alpha-carotene (see the accompanying drawings which show a formula therefor)

Auroxanthin

Antheraxanthin

Eloxanthin

Eschscholtz-xanthin

Flavoxanthin

Violaxanthin

Zeaxanthin

Ketonic

Astacene	(di-hydroxy and di-keto)
Astaxanthin	(di-hydroxy and di-keto)
Capsanthin	(dihydroxy and mono-keto)
Capsorubin	(dihydroxy, di-keto)
Canthaxanthin	(which can be in a di-keto or di-hydroxy form)
Fucoxanthin	(tetra hydroxy, di-keto)
Rhodoxanthin	(di-keto)

It is, of course, well known that Keto moieties can enolize to give hydroxy groups.

More preferably, the hydrophilic carotenoid used in the invention is a di-hydroxy carotenoid, especially lutein.

Also, in carrying out the invention the active hydrophilic carotenoid may be used together with other active agents. Amongst such other active agents there may be mentioned, for example, the following, namely:

Another carotenoid such as:

Lycopene, or

Alpha, beta, gamma or delta carotene.

or one or more of the following antioxidants or anti-inflammatory agents, namely:

Vitamin A

Vitamin C

Vitamin E (α-tocopherol and other active tocopherols)

Selenium

Copper

Zinc

Manganese

Ubiquinone (Coenzyme Q10)

Aspirin

Salicylic acid

2,3-dihydroxy benzoic acid

2,5-dihydroxy benzoic acid

Use of a mixture with α-tocopherol and/or aspirin is

especially preferred since it is believed that such a mixture affords a synergistic effect, especially with lutein.

In carrying out the invention the amount of hydrophilic carotenoid e.g. lutein, used will vary depending on the effect sought. Generally speaking, however, the hydrophilic carotenoid as active agent may be used in a dosage regimen between about 0.5 and about 50 mg per day, typically about 1 to about 30 mg.

The hydroxycarotenoids are partially destroyed in the gastrointestinal tract by oxidation. By adding Vitamin E and/or Vitamin C this process is inhibited and more hydroxycarotenoid is absorbed. The inhibitor may be included as part of a composition according to the invention or administered separately.

A unit dosage form such as say a 750 mg tablet or say an 800 mg capsule to be used on a one-a-day basis may contain between about 0.1% and about 4% by weight of lutein and other ingredients may comprise:

Beta carotene	about 2 to about 20 mg e.g. about 5 mg
Vitamin A	about 400 to about 600 RE e.g. about 500 RE
Vitamin C	about 75 to about 250 mg e.g. about 100 mg
Selenium	about 80 to about 120 mcg e.g. about 90 mcg
Copper	about 2 to about 4 mg e.g. about 3 mg

Zinc	about 10 to about 20 mg e.g. about 15 mg
Aspirin	about 10 to about 150 mg e.g. about 50 mg
Salicylic acid	about 10 to about 150 mg e.g. about 50 mg
2,3-dihydroxy benzoic acid	about 10 to about 150 mg e.g. about 50 mg
2,5-dihydroxy benzoic acid	about 10 to about 150 mg e.g. about 50 mg
Manganese	about 2 to about 5 mg e.g. about 4 mg
Ubiquinone (Coenzyme Q10)	about 10 to about 100 mg e.g. about 50 mg
Carrier	about 150 to about 250 mg e.g. about 175 or about 200 mg.

In addition to the above aspects, the invention includes the use of a hydrophilic carotenoid, especially a mono- or di-hydroxy carotenoid such as lutein or an ester thereof, a ketonic carotenoid, or a mixture of the same for the manufacture of a food supplement or medicament for the prevention or treatment of disease involving oxidation of lipids, lipoproteins, proteins or DNA.

Furthermore, the invention includes a process for the manufacture of a food supplement or medicament for use in the treatment of disease involving oxidation of lipids, lipoproteins, proteins or DNA which process comprises formulating a hydrophilic carotenoid, especially a mono- or di-hydroxy carotenoid such as lutein or an ester thereof, a ketonic carotenoid, or a mixture of the same for use in such treatment.

Still further, the invention includes a method for the prevention or treatment of cardiovascular or cerebrovascular disease, cancer, diabetes, rheumatoid arthritis, Parkinson's disease, Down's syndrome, Alzheimer's disease or cataracts or for delaying other age-related changes which method comprising administering an effective amount of a hydrophilic carotenoid, especially a mono- or di-hydroxy carotenoid such as lutein or an ester thereof, a ketonic carotenoid, or a mixture of the same as active agent.

The following Examples are intended to illustrate the invention.

EXAMPLE 1

An epidemiological study was conducted to compare relevant factors as between the cities of Belfast and Toulouse in relation to coronary heart disease (CHD).

The incidence of CHD in Belfast is 4.5 times greater in men than in Toulouse, and 8.0 times greater in women than in Toulouse. This is despite the fact that in both cities, major risk factors such as total plasma cholesterol, total fat intake, saturated fat intake, alcohol intake, smoking habits, body weight, and blood pressure are the same. Gey (1990, 1993) has suggested that differences between different European cities in CHD is due to the different intakes of alpha tocopherol (vitamin E), vitamin C, vitamin

A, and beta-carotene, and that plasma levels of these micronutrients are indicative of risk because of the high antioxidant activity of these nutrients. No information on plasma carotenoids (other than alpha and beta-carotene), is available in relation to the incidence of CHD in different populations.

A random selection was made of 171 people aged 45 years to 65 years in Belfast (90 men and 81 women) with a similar sample of 211 people from Toulouse (101 men and 110 women). After an overnight fast 20 ml of blood was taken from the cubital vein, into EDTA, and the separate plasma stored at -80°C until analysis by HPL chromatography. As shown in Table I below there was no meaningful difference between the two groups of subjects in the plasma levels of ascorbate (vitamin C), alpha-tocopherol (vitamin E), retinol (vitamin A), and beta-carotene and lycopene (non hydroxy carotenoids). However, the plasma levels of lutein and α-and β-cryptoxanthin were substantially increased in the Toulouse subjects relative to the Belfast group.

TABLE I

PLASMA, VITAMINS AND CAROTENOIDS IN TWO CITIES WITH A LARGE DIFFERENCE IN THE INCIDENCE IN CORONARY HEART DISEASE ++

COMPARISON OF BELFAST AND TOULOUSE STUDIES ALL SUBJECTS: ALL AGES MALES AND FEMALES

	Belfast (n = 171)	Toulouse (n = 211)	P	
Ascorbate (μmol/L)	34.1 ± 1.2 (167)	32.9 ± 1.0 (208)	0.3402	NS
Retinol (μmol/L) ø	1.97 (1.89 - 2.06) (167)	1.85 (1.77 - 1.92) (206)	0.0262	*
Lutein (μmol/L) ø	0.25 (0.23 - 0.27) (167)	0.54 (0.51 - 0.57) (206)	< 0.0001	***
y-Tocopherol (μmol/L) ø	2.15 (2.00 - 2.31) (167)	1.41 (1.32 - 1.50) (206)	< 0.0001	***
α-Cryptoxanthin (μmol/L) ø	0.06 (0.05 - 0.07) (167)	0.10 (0.09 - 0.11) (206)	0.0001	***
α-Tocopherol (μmol/L) ø	28.6 (27.2 - 30.1) (167)	27.3 (26.5 - 28.1) (206)	0.1031	NS
β-Cryptoxanthin (μmol/L) ø	0.10 (0.09 - 0.12) (167)	0.23 (0.21 - 0.25) (206)	0.0001	***
α-Carotene (μmol/L) ø	0.08 (0.07 - 0.09) (167)	0.12 (0.11 - 0.13) (206)	< 0.0001	***
β-Carotene (μmol/L) ø	0.36 (0.32 - 0.40) (167)	0.39 (0.35 - 0.43) (206)	0.3666	NS
Lycopene (μmol/L) ø	0.38 (0.34 - 0.42) (167)	0.38 (0.35 - 0.41) (206)	0.9409	NS

Results are mean ± SEM (n), or mean (95% confidence interval) (n)
ø Results were log transformed prior to analysis
P is the significance determined by two-tailed unpaired t-test
++ Belfast has a much higher incidence of CHD than Toulouse
+ The lutein estimated contains a small proportion of Zeaxanthin

As can also be seen from Table I, y-tocopherol (with vitamin E activity) was increased 50% in Belfast compared with Toulouse. On the other hand the carotenoids lutein, alpha and beta-cryptoxanthin were increased by 100% in Toulouse compared with Belfast.

The largest change in concentration was lutein. Alpha carotene was increased by 50% in Toulouse, but its concentration was very small (1/5th of that of lutein).

The results indicate that there is a major difference in the plasma concentration of hydroxy carotenoids (lutein, and alpha- and beta-cryptoxanthin) between the two cities. Furthermore, the higher plasma concentration of hydroxy carotenoids in the Toulouse subjects correlates with the lower incidence of CHD in Toulouse as compared with Belfast. This predicts that supplementing the populations susceptible to CHD with hydroxy carotenoids in accordance with the invention would prevent CHD.

One of the major protagonists of the dietary antioxidant hypothesis has been Gey [British Medical Bulletin (1993), Vol 49, No 3] who proposed that vitamins A, C and E and β-carotene were antioxidants protective against CHD, and suggested optimum therapeutic ranges (μmol/L) for Vitamin A (2.2 to 2.8) Vitamin C (40 - 50) and Vitamin E (28 to 30) and β-carotene (0.4 to 0.5). The concentrations of all the above mentioned vitamins were the same in Belfast as Toulouse, and except for Vitamin C, which was only 75% of

the optimum, both cities are within the therapeutic ranges. Gey has especially promoted the idea that Vitamin E was the most important, even though his own data shows no difference between Belfast and Toulouse, and his values are in agreement with those tabulated above in Table I. In a recent primary prevention trial in 29,000 smokers, subjects were given 20 mg/day β-carotene and/or 50 mg Vitamin E for 5 year, β-carotene increased mortality from cancer of the lung and CHD. Vitamin E had no effect on mortality. This major study [OP Heinomen et al, New England J Med, Mass Med Soc, Volume 330, No 15, 1029 et seq] unfortunately did not substantiate the proposed merits of β-carotene or Vitamin E in disease prevention. The increase in mortality with β-carotene is unexpected but not unexplainable: β-carotene in large doses inhibits the absorption of other carotenoids which may be more effective antioxidants.

At the atmospheric pressure which would exist in the lung, β-carotene has been shown to be a pro-oxidant compared with lutein and lycopene which are antioxidants.

EXAMPLE 2

It is also possible that lutein may protect LDL directly from oxidation, due to its presence in the lipoprotein particles. β-carotene does not prevent copper mediated oxidation of LDL but the reason lutein might be more potent is that the hydroxy groups render the molecule more hydrophillic and lutein is more likely to be present on the

surface of LDL.

The effect of lutein on copper-initiated oxidation of LDL in vitro was examined as follows:-

Plasma was incubated for 3 hours with 20 and 50 μ mol of lutein per litre of plasma at 37°C. As the incubations each required 5 ml of plasma the amounts of lutein actually used were 54 and 135 μg. Following incubation LDL were isolated and the oxidation was initiated with copper sulphate. Diene conjugate formation was monitored at 234 nm at 28°C. Results showed a significant increase in LDL lutein concentration from 0.078 in a control to 0.89 nmol mg LDL (mass/ml). This increase in LDL lutein concentration raised the lag phase from 104 minutes in the control to 217 minutes in supplemented LDL. This suggests that lutein may be potentially as important a lipophilic antioxidant as tocopherol in preventing the oxidative modification of LDL.

EXAMPLE 3

Three normal volunteers were given 30 mg/day lutein for one week, and their plasma separated by ultra centrifuge into high density lipoprotein (HDL) and low density lipoprotein (LDL) fractions. These were analyzed for lutein and cholesterol.

As shown in Table II below, the lutein:cholesterol ratio

(nmole/mmole) was greater, prior to dosing, in HDL. After dosing, the lutein concentration rose much more in the HDL than LDL fraction such that the ratio of lutein/cholesterol in HDL was over three fold greater than in LDL.

TABLE II

EFFECT OF LUTEIN (30 MG/DAY) FOR ONE WEEK ON THE LUTEIN CONTENT OF LIPOPROTEIN FRACTIONS

	n/mole lutein / m/mole cholesterol			
	LDL		HDL	
Subject No	Before	After	Before	After
1	2.66	6.36	5.71	14.3
2	2.40	5.45	4.11	20.7
3	0.74	4.55	3.72	18.9
Mean	1.93	5.45	4.51	18.0

People in Toulouse had elevated HDL cholesterol compared with Belfast. This is consistent with many other studies in which this lipid parameter is high in populations with low CHD.

Although there are many mechanisms by which HDL acts as an antioxidant for LDL by providing a sink for lipoperoxides. It is possible that one of the active components of HDL might be lutein which is present in higher concentrations than the other carotenoids and which has been demonstrated to possess peroxy radical scavenging activity at

atmospheric pressure, in contrast to β-carotene which is inactive under these conditions.

EXAMPLE 4

It is believed that the long term symptoms of diabetes are due to reactive oxygen species. Diabetes is also associated with an increased risk of coronary heart disease (CHD). The plasma concentrations of some antioxidants in diabetics and healthy subjects as controls matched for age and sex were measured.

Blood was collected and the plasma analyzed for hydroxy carotenoids by HPLC. The results were log transformed prior to statistical analysis by a two tailed t-test.

As shown in Table III, levels of lutein was decreased by 30% in the diabetic subjects relative to the controls (but there was no detectable change in the level of cryptoxanthins). The results indicate that decreased plasma lutein is a risk factor for CHD, and suggest that supplementation with lutein and/or other hydroxy carotenoids would have a beneficial effect in terms of reducing CHD risk in diabetics.

TABLE III

	CONTROLS n	Mean (95% C.I.)	DIABETICS n	Mean (95% C.I.)	P	
Ascorbate (μmol/L)	10	59.8 (44.9-79.7)	10	41.9 (29.1-60.2)	0.098	NS
α-carotene (μmol/L)	10	0.106 (0.082-0.135)	10	0.049 (0.032-0.075)	0.002	**
β-carotene (μmol/L)	10	0.472 (0.316-0.705)	10	0.231 (0.140-0.382)	0.022	*
Lutein (μmol/L)	10	0.433 (0.336-0.558)	10	0.307 (0.243-0.389)	0.037	*
Lycopene (μmol/L)	10	0.707 (0.547-0.912)	10	0.369 (0.236-0.576)	0.010	*
Retinol (μmol/L)	10	2.61 (2.01-3.39)	10	2.05 (1.62-2.60)	0.137	NS
α-tocopherol (μmol/L)	10	35.3 (29.3-42.4)	10	30.2 (22.1-41.3)	0.348	NS
Mono. Cu (nmol/10°cells)	25	11.3 (9.4-13.7)	21	8.5 (7.3-9.9)	0.022	*
Mono. Zn (nmol/10°cells)	25	158 (137-182)	22	176 (134-231)	0.464	NS
Gran. Cu (nmol/10°cells)	25	4.7 (3.6-6.1)	20	3.4 (2.5-4.6)	0.099	NS
Gran. Zn (nmol/10°cells)	25	109 (93-128)	22	100 (79-125)	0.481	NS
Plasma Cu (μmol/L)	26	15.6 (14.2-17.1)	88	13.0 (12.3-13.8)	0.003	**
Plasma Zn (μmol/L)	26	12.5 (11.9-13.1)	88	10.5 (10.1-10.9)	<.001	***
RBC SOD (units/g Hb)	53	1319 (1224-1421)	51	1089 (1018-1166)	0.002	**

n = number of subjects * P < 0.05 ** P < 0.01 *** P < 0.001

Mono = mononuclear Gran = granulocyte

Other antioxidants such as alpha- and beta carotene and lycopene were also reduced. The copper was reduced in mono-nuclear cells and plasma Zinc was reduced in plasma. The copper dependent enzyme SOD (Superoxide dismutase) was reduced in red blood cells.

On the basis of these results it is now possible to formulate an antioxidant preparation which would be especially suitable for diabetics as follows:-

Ingredients per capsule	Label claim	mg/capsule
Lycopene (5% solution in oil)	5 mg Lyc	110
Carotene oil	5 mg BC	18
Lutein ester	5 mg LUT	50
Vitamin C	100 mg C	105
Mixed tocopherols (1000 lu/gm)	100 mg E	150
Selenium yeast (1000 mcg/gm)	90 mcg Se	90
Copper amino acid Complex	3 mg Cu	100
Zinc gluconate	15 mg Zn	117
Vegetable shortening		50
Beeswax		23
Lecithin		22
Soyabean oil		75
		910

EXAMPLE 5

A mixture was prepared to the formulation set forth above in Example 4 but with the addition of 5 mg/capsule alpha carotene.

EXAMPLE 6

A capsule was prepared using the following ingredients by simple admixture and routine encapsulation:-

Ingredients per capsule	Label Claim	mg per Capsule
Lutein Ester	20 mg Lutein	150
Lecithin		25
Soya Bean Oil		100

EXAMPLE 7

A capsule was prepared using the following ingredients by simple admixture and routine encapsulation:-

Ingredients per capsule	Label Claim	mg per Capsule
Vitamin C (Ascorbic Acid)	150 mg	160
α - Tocopherol	100 mg	110
Lutein Ester	15 mg Lutein	90
Lecithin		25
Soya Bean Oil		75

EXAMPLE 8

The procedure of Example 7 was repeated except that 30 mg of Coenzyme Q10 was included in the mixture and the mixture encapsulated.

EXAMPLE 9

A size 12 oval capsule of nominally 800 mg weight was prepared from the following ingredients by simple admixture and routine encapsulation:-

Ingredients per capsule	Label Claim	mg per Capsule
Vitamin A Palmitate 1500 iu/gm	500 RE	1.277
Carotene Oil	15 mg BC	52.5
Lutein Ester*	7.5 mg Lutein	50
Vitamin C (Ascorbic Acid)	100 mg	105
Mixed Tocopherols 1000 iu/gm	100 mg	149
Selenium Yeast 1000 mcg/gm	90 mcg	90
Copper Gluconate to give	3 mg Cu	22.26
Zinc Gluconate to give	15 mg Zn	117
Manganese Gluconate to give	4 mg Mn	36.4
Vegetable Shortening		56
Beeswax		23
Lecithin		22
Soya Bean Oil		75.563
		800

* concentrated lutein esters with an E (1%, 1 cm) of 300 to 340 at 453 nm in chloroform - corresponds to a pure lutein content of 12 to 14.4%.

EXAMPLE 10

A dry powder formula diet composition was prepared by mixing 150 mg of lutein ester per day with a Cambridge Diet (The Cambridge Diet is a Registered Trade Mark) product obtained from Cambridge Health Plan Limited, Norwich, England.

EXAMPLE 11

Example 7 was repeated a total of sixteen times, in each case a hydroxycarotenoid from the compounds listed below being included in the encapsulated mixture in an amount of 15mg in substitution for lutein ester:-

β-cryptoxanthin	p-cryptoxanthin	auroxanthin
violaxanthin	flavoxanthin	eloxanthin
antheraxanthin	erschscholtz-xanthin	astacene
astaxanthin	capsanthin	capsorubin
canthaxanthin	flucoxanthin	rhodoxanthin
zeaxanthin		

EXAMPLE 12

Eight Adults were given orally a daily capsule containing

the formulation of Example 9 with an evening meal for 4 weeks. Blood (10 ml) was taken after an overnight fast, before, 2 weeks after and 4 weeks after taking the capsule. Plasma, vitamins and carotenoids were analyzed by HPLC as shown in Table IV. The mean concentration of lutein was increased from 0.309 to 0.667 μmol/L after 4 weeks administration. In Example 1, the mean concentration of lutein in people from Toulouse was 0.54 μmol/L. Thus the capsule was able to provide in a daily therapeutic dose sufficient to bring the concentration of lutein into a beneficial range. Rather surprisingly retinol (Vitamin A) was unchanged, but the expected increases in plasma, β-carotene and α-tocopherol (also present in the capsule) occurred.

TABLE IV

EFFECT OF A SPECIFIC FORMULATION ON FAT SOLUBLE VITAMINS (RETINOL, TOCOPHEROLS) AND PLASMA CAROTENOIDS

Parameter µmol/L	Week 0 Mean		Std Dev	Week 2 Mean		Std Dev	Week 4 Mean		Std Dev
Lutein	0.309	±	0.105	0.594	±	0.244**	0.663	±	0.18***
Lycopene	0.668	±	0.345	0.640	±	0.308	0.517	±	0.195
α-Carotene	0.0815	±	0.0423	0.0914	±	0.0351	0.0828	±	0.0379
β-Carotene	0.473	±	0.538	1.07	±	0.584*	1.12	±	0.651**
α-Cryptoxanthin	0.100	±	0.0628	0.125	±	0.081	0.132	±	0.0967
β-Cryptoxanthin	0.3409	±	0.322	0.352	±	0.318	0.324	±	0.304
α-Tocopherol	26.3	±	8.25	38.7	±	9.68***	37.1	±	8.54**
γ-Tocopherol	1.36	±	0.670	0.707	±	0.248*	0.691	±	0.163*
Retinol	1.90	±	0.513	2.034	±	0.534	1.97	±	0.505

* $p < 0.05$
** $p < 0.01$
*** $P < 0.001$

EXAMPLE 13

A capsule was prepared using the following ingredients by simple admixture and routine encapsulation:-

Ingredients per capsule	Label Claim	mg per capsule
α-Tocopherol	50 mg	55
Aspirin	50 mg	55
Lutein ester	10 mg Lutein	60
Lecithin		25
Soya Bean oil		75
		270

In the above example, Aspirin can be replaced by salicylic acid, 2,3-dihydroxy benzoic acid or 2,5-dihydroxy benzoic acid.

While the invention has been described above in various specific details, it will be appreciated that numerous and various modifications may be made. Thus, for example, the ingredients can be in various other proportions, of which the above specifically recited are examples only.

Claims

1. A hydrophillic carotenoid antioxidant (HCA) for use as a pharmaceutical.

2. Use of an HCA for the preparation of a medicament for use in the treatment by therapy or prophylaxis of a subject to relieve or reduce risk of contraction of a disease having an oxidation mechanism.

3. Use of an HCA for the preparation of a medicament for use in the treatment by therapy or prophylaxis of a subject to relieve or reduce risk of contraction of a disease involving oxidation of body lipids, proteins or DNA.

4. Use of an HCA for the preparation of a medicament for use in the treatment by therapy of prophylaxis of a subject to relieve or reduce risk of contraction of a disease involving oxidation of bodily lipoproteins.

5. Use of an HCA for the preparation of a medicament for use in the treatment by therapy or prophylaxis of a subject to relieve or reduce risk of contraction of cardiovascular or cerebrovascular disease, cancer, cataracts, diabetes, rheumatoid arthritis, Parkinson's disease, Down's syndrome, Alzheimer's disease or other age-related diseases.

6. Use of an HCA for the preparation of a medicament for

use in the therapy or prevention of coronary heart disease.

7. Use as claimed in any preceding claim wherein the HCA is a hydroxycarotenoid.

8. Use as claimed in Claim 7 wherein the HCA is a monohydroxy carotenoid as set forth by name in the following list:-

8.1 Beta cryptoxanthin
8.2 Alpha cryptoxanthin
8.3 Anhydrolutein.

9. Use as claimed in Claim 7 wherein the HCA is a dihydroxy carotenoid as set forth by name in the following list:-

9.1 Lutein
9.2 Zeaxanthin
9.3 Auroxanthin
9.4 Violaxanthin
9.5 Flavoxanthin
9.6 Eloxanthin
9.7 Antheraxanthin
9.8 Eschscholtz-xanthin

10. Use as claimed in Claim 7 wherein the HCA is a ketonic carotenoid as set forth by name in the following list:-

10.1 Astacene

10.2 Astaxanthin

10.3 Capsanthin

10.4 Capsorubin

10.5 Canthaxanthin

10.6 Fucoxanthin

10.7 Rhodoxanthin

11. Use as claimed in any one of Claims 2 to 10 wherein the HCA is used in combination with another carotenoid.

12. Use as claimed in Claim 11 wherein the other carotenoid is lycopene or alpha, beta, gamma or delta carotene.

13. Use as claimed in any one of Claim 2 to 12 wherein the HCA is used in combination with another antioxidant or anti-inflammatory agent.

14. Use as claimed in Claim 13 wherein the other antioxidant is vitamin A, vitamin C, vitamin E, selenium, copper, zinc, manganese or ubiquinone (Coenzyme Q10), aspirin, salicylic acid, 2,3-dihydroxy benzoic acid or 2,5-dihydroxy benzoic acid.

15. A composition for use as a pharmaceutical and comprising an HCA together with a pharmaceutically

acceptable carrier or diluent.

16. A composition for use as a food supplement and comprising an HCA together with an acceptable carrier or diluent.

17. A composition as claimed in Claim 15 or Claim 16 in unit dosage form.

18. A composition as claimed in Claim 17 wherein the composition is in tablet, capsule, powder, solution or suspension unit dosage form.

19. A method of medical treatment which method comprises administering to a subject suffering from or at risk of contracting a disease having an oxidation mechanism, an HCA for the purposes of therapy or prophylaxis.

20. A method for the medical treatment of subjects suffering from coronary heart disease or at risk of contraction thereof, which method comprises administering to the subject an HCA.

21. A method of antioxidative in vitro treatment of lipoproteins which method comprises administering to a mammalian subject an HCA.

22. A method for the medical treatment of subjects

suffering from cardiovascular or cerebrovascular disease, cancer, cataracts, diabetes, rheumatoid arthritis, Parkinson's disease, Downs Syndrome, Alzheimer's disease or other age-related diseases, which method comprises administering to the subject an HCA.

23. A hydrophilic carotenoid antioxidant for use in the manufacture of a medicament for the treatment of a disease having an oxidation mechanism.

Patents Act 1977 Examiner's report to the Comptroller under Section 17 (The Search report) 32	Application number GB 9412938.4
Relevant Technical Fields (i) UK Cl (Ed.M) A5B (BHA, BJA) (ii) Int Cl (Ed.5) A61K 31/07	Search Examiner J F JENKINS Date of completion of Search 16 SEPTEMBER 1994
Databases (see below) (i) UK Patent Office collections of GB, EP, WO and US patent specifications. (ii) ONLNE DATABASE: CAS-ONLINE	Documents considered relevant following a search in respect of Claims :- 1 TO 18 AND 23

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X:	Document indicating lack of novelty or of inventive step.	P:	Document published on or after the declared priority date but before the filing date of the present application.
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A:	Document indicating technological background and/or state of the art.	&:	Member of the same patent family; corresponding document.

Category	Identity of document and relevant passages		Relevant to claim(s)
X	GB 1323800	(HOFFMANN-LA ROCHE) see Examples 1 to 3	1,10-12 15 and 18
X	EP 0385335 A2	(NISSHIN FLOUR) see page 2 lines 45-47, Examples 5 and 6, Claim 8	1,7,9-12, 15-18
X	WO 91/06292 A1	(DANOCHE-MO) see Examples 6 and 7	1,10,15, 17 and 18
X	WO 85/03225 A1	(L'OREAL) see Claim 2	1,10,15
X	US 4957681	(KLIMESCH) see Examples 31 and 32	1,10,15
X	US 4929774	(FUKAMACHI) see working Example 6-1	1,10,15
X	Chemical Abstracts 119:56200 and DE-4141351-A (SCHNEIDER et al)		1,10,15 and 16
X	Chemical Abstracts 113:65273 & JP-020049091 (UCHIUMI et al)		1,10,15
X	Chemical Abstracts 109:215833 & Khim. Prir. Soedin, 4, pages 524-9 (1988) (MATIS et al)		1,7,9,10 15
X	Chemical Abstracts 107:161484 & Acta Pharm. Hung. 57 (3-4) pages 153-8 (1987) (PAPAY et al)		1,7,9,15

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